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An *In Vitro* Analysis of Egg Mortality in *Cancer anthonyi:* The Role of Symbionts and Temperature

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Abstract. Several symbionts occur on crab eggs. These symbionts, and the effect of temperature, have been implicated as causal mechanisms of egg mortality in *Cancer magister.* The contribution of three symbionts (a fungus, *Lagenidium callinectes;* bacteria, *i.e., Leucothrix* sp.; and a nemertean worm, *Carcinonemertes epialti*) to egg mortality on *Cancer anthonyi* were investigated *in vitro* using a multifactorial experimental design at four different temperatures. The nemertean worm was found to contribute most to egg mortality on the individual crab and at the crab population level because its prevalence was high (>95%) and it had a relatively constant feeding rate. *Lagenidium callinectes* caused from 20–75% egg mortality on individual crabs but its prevalence was nil. A *Lagenidium*-like fungus had a low prevalence (2.6%) and was not associated with egg mortality. While bacteria were omnipresent, they were found to cause negligible crab egg mortality. Few significant interactions were observed between the symbionts. Temperature had a significant effect on worm feeding rates, worm oviposition, and fungal attack rates. At low temperatures (4 and 10°C), symbionts killed fewer eggs than at higher temperatures (15 and 20°C). Extreme temperatures (4 and 20°C) caused variable degrees of egg mortality, yet some eggs survived at these temperatures. Temperature also had a profound effect on egg development. At 20°C, eggs developed almost twice as fast as those at 10°C. Development appeared to stop at 4°C.

Introduction

Several symbionts have recently been reported to cause egg mortality in the broods of the Dungeness crab, *Cancer magister.* A nemertean worm, *Carcinonemertes errans,* eats the eggs of *C. magister* (Wickham, 1978, 1979a, 1980; Roe, 1984). At high densities, it may account for the high incidence of egg mortality observed on *C. magister* (Wickham, 1979a, 1980). Other symbionts have also been implicated as possible agents of crab egg mortality. Bacterial fouling (*e.g., Leucothrix* sp.) of the egg masses of crabs is highly correlated with worm number, developmental stage of the crab eggs, and egg mortality (Fisher, 1976; Fisher and Wickham, 1976; Wickham, 1979a). The fungal pathogen, *Lagenidium callinectes,* has been reported in cultures of larval *Cancer magister* (Armstrong *et al.,* 1976), but its presence in the clutch of *C. magister* has not been well established. The prevalence of *L. callinectes* on the eggs of the blue crab, *Callinectes sapidus,* is correlated with the prevalence of *Carcinonemertes carcinophila* (Rogers-Talbert, 1948).

Nemertean worms, various fungi, and numerous bacteria may all be found on the same crab (Fisher and Wickham, 1976; Shields, pers. obs.). Therefore, it has been difficult to determine if the symbionts act separately, or in conjunction, to produce egg mortality. Hamilton (1984) showed that crab egg mortality may result from either fungal infection or nemertean infestation. However, fungi were present in her egg cultures containing *Carcinonemertes errans.* Fisher (1976) experimentally increased bacterial fouling by increasing the available nutrients present in seawater. In control treatments with antibiotics, egg mortality decreased. However, *C. errans* may have been present in the broods of these experimental crabs (Wickham, 1979a). The contribution of the other symbionts to brood loss has not been examined.

The cyclic fluctuations in *Cancer magister* populations have received much empirical and theoretical attention (*e.g.,* Botsford and Wickham, 1978; Wild, 1983;

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Hankin, 1985). High temperature (Mayer, 1973; Wild, 1983) and nemertean egg predation (Wickham, 1979a, b) have been invoked as possible contributory factors in the decline and non-recovery of the central California population of *C. magister*. While temperature variation clearly influences the rate of egg development and egg mortality, the role of *Carcinonemertes errans* in producing egg mortality has been incompletely assessed (Wild, 1983).

To date there have been no quantitative studies of crab eggs exposed to only *L. callinectes*, bacteria (*Leucothrix* sp.), or *Carcinonemertes* species. We used a multifactorial experimental design to determine the relative contributions *in vitro* of each symbiont (*Carcinonemertes epialti*, *Lagenidium callinectes*, and *Leucothrix* sp.) to the egg mortality of *Cancer anthonyi*. *Cancer anthonyi* was used throughout this study as it is locally abundant and breeds year-round (Reilly, 1987). The susceptibility of eggs in different developmental stages was also examined. In addition, the effect of temperature on egg mortality and development, and on symbiont behavior was investigated.

Materials and Methods

Ovigerous crabs were collected at nearshore depths of 10–100 m by a commercial fisherman. Crabs were trapped from the Santa Barbara Channel, between Summerland and Gaviota, California, and maintained at ambient seawater temperatures (12–17°C) in 280 l flow-through fiberglass aquaria. Twenty-five crabs were used in the study.

A single ovigerous pleopod was excised from crabs bearing embryos in various stages of development. The developmental stages of embryogenesis (EDS-egg developmental stage) were assessed as per Shields (1987). Egg-bearing setae were removed from the pleopod and placed in UV-filtered seawater (2–35 μm filters, one ultraviolet-light filter, Rainbow Plastics, Filter Division, El Monte, California). Samples consisting of 80–250 eggs attached to individual and intertwined setae were counted while using a dissecting microscope, and the number of dead eggs and their apparent cause of death (*e.g.*, mechanical disruption, infertility, etc.) were noted. After counting, the sample was washed in UV-filtered seawater containing 1.0% bleach for 2–4 minutes to kill or remove microorganisms, and placed in a 35 × 10 mm plastic petri dish with 3.0 ml of UV-filtered seawater containing, in some treatments, antibiotics [500 mg each of penicillin-G and streptomycin sulfate per liter (Sigma Co.)]. The egg samples were then exposed to various pathogens detailed below and were placed in an incubator at 4, 10, 15, or 20°C. Five to ten replicates of egg samples were examined in each treatment.

Various egg samples were exposed to male or female *Carcinonemertes epialti*. Worms were removed from the excised pleopods of crabs by vigorous agitation in 200–300 ml seawater. Worms were taken from crabs whose eggs were in similar stages of development to those in the experimental treatments. Individual worms were then pipetted into a petri dish where they were sexed prior to being placed with the previously counted egg samples. Egg predation by the worms, worm behavior in the petri dish, and the number and time to hatching of worm egg strings were noted every two days for the duration of each experiment. Egg production was measured as the mean number of egg strings produced by each female worm at each stage of crab egg development. The egg predation rate was determined for each worm by dividing the number of eggs eaten by ten—the number of days in each experiment.

A pure culture of *Lagenidium callinectes* was acquired from the American Type Culture Collection (#24973 ATCC). Cultures of *L. callinectes* were grown in liquid Kazama's modified Vishniac medium (KMV: 1.0 g glucose, 1.0 g gelatin hydrolysate, 0.1 g bacto-peptone, 0.1 g yeast extract, 1.0 l seawater, 1.0 g agar). Cultures were maintained both with and without antibiotics (500 mg each of penicillin-G and streptomycin sulfate per liter) at 15 and 20°C. Zoospores and resting spores from 2- to 6-week-old cultures of *L. callinectes* were counted using a hemocytometer (Levy counting chamber). Three replicates of the culture were counted and the appropriate dilutions were made to give an estimated density of 150 zoospores/ml. Various egg samples were then exposed to 1.0 ml of the diluted *L. callinectes* culture. To examine the effect of initial zoospore density on egg mortality, exposures of 15, 150, and 1500 zoospores/ml were examined at 15°C.

The prevalence of *L. callinectes* from wild *Cancer anthonyi* was examined. Isolation of *L. callinectes* was attempted for monthly or bimonthly samples of *C. anthonyi* eggs. Egg samples were removed from crabs and placed in sterile petri dishes containing UV-filtered seawater. Fresh samples were examined for the presence or absence of bacteria and fungi. After three days, the samples were again observed for symbionts. Using sterile pipettes, the samples were then streaked onto sterile agar (KMV with antibiotics) petri dishes. Seawater controls were also cultured. After an additional 3–5 days, the plates were examined for fungal symbionts.

Cultures of *Leucothrix* sp. and other bacteria were isolated on KMV from crab egg clutches. No attempt was made to isolate individual species of bacteria. The cultures were used only if *Leucothrix* sp. was present. Filamentous bacteria and the typical "fingerprint" colonies of *Leucothrix* sp. (Johnson *et al.*, 1971) were used to indicate the presence of usable, experimental cultures.

Counts of bacteria were made in the same manner as the fungal counts. A density of 75,000 bacteria/ml was used in the study. Egg samples were inoculated in the same manner as the those exposed to the fungus.

Egg samples were exposed to each symbiont alone and to each combination of symbionts. A separate treatment of eggs exposed to antibiotics and diluted KMV medium was a control for the use of these products. An additional treatment of eggs that were not exposed to bleach, antibiotics, and KMV, was an additional control for the various manipulations. Crab egg mortality was assessed every two or three days for ten days.

Statistical procedures are described in Sokal and Rohlf (1981). Arcsin transformations were used when appropriate, *i.e.*, to normalize ratios and percentages. A value of $P < 0.05$ was significant.

Results

General observations

Eggs killed by worms were either wholly or partially devoid of yolk material; their egg coats were torn. Eggs killed by the fungus became opaque. Infected embryonic tissue was characterized by black and brown spots of pigment. Infected eggs initially appeared fuzzy. Later, fungal exit tubes protruded from the surface of the eggs. In later stages of development, embryos killed by the fungus were often misshapen, and roughly ellipsoid in form. Such an appearance presumably resulted from reduced internal pressure upon zoospore penetration. Misshapen eggs appeared to have an intact internal coat surrounding the embryo. Eggs killed by bacteria eventually became opaque and possessed a filamentous border.

At each temperature, some eggs were developmentally retarded. Many of these eggs never developed further. The cause of this phenomenon is unknown. Data on asynchronous development of the embryos are presented below.

Carcinonemertes epialti

No significant differences were detected between egg predation rates for worms in the presence or absence of bacteria (worms alone, and worms with bacteria). These treatments were, therefore, combined for the analysis. The feeding rates for both sexes were combined for those treatments maintained at 4°C since little egg predation occurred at that temperature. Only 10 of the 75 worms examined at 4°C ate eggs.

The predation rate of male *C. epialti* varied with temperature. Male worms had a significantly lower predation rate at 4 and 10°C than at the higher temperatures (Fig. 1A: ANOVA, Sidak's inequality, $P < 0.05$). However, there were no significant differences between predation rates for treatments at 15 and 20°C (ANOVA, Sidak's inequality, $P > 0.05$). The predation rates of male worms were not significantly different between the stages of crab egg development within each temperature regime.

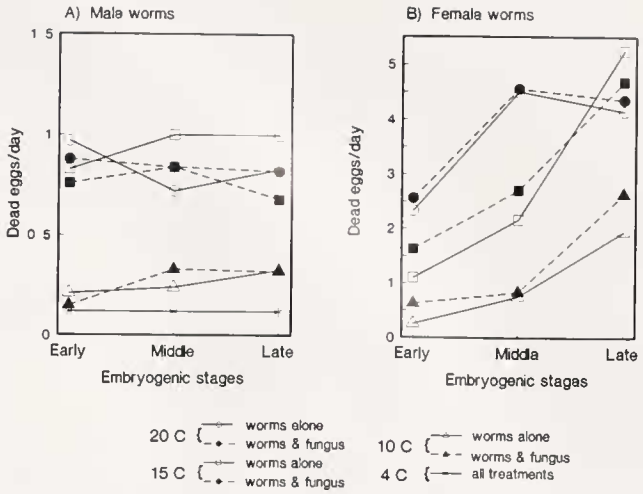

Figure 1. Predation rates of *Carcinonemertes epialti* feeding on the eggs of *Cancer anthonyi* at different temperatures. Early, middle, and late refer to stages in crab egg development. (A) Predation rates of male worms; (B) Predation rates of female worms. N ≥ 5 replicates in all treatments.

Female worms had a much higher predation rate than male worms (Fig. 1B). The predation rate of female worms increased with temperature and with the developmental stages of the crab eggs (Fig. 1B). At 4 and 10°C, the predation rate at each stage of egg development was significantly less than those rates at the higher temperatures (ANOVA, Sidak's inequality, $P < 0.05$). Female worms fed at significantly lower rates during the early and middle stages of egg development at 10 and 15°C, than during the latter part of embryogenesis (ANOVA, Sidak's inequality, $P < 0.05$). At 20°C, however, predation rates were highest during the middle of embryogenesis. They were significantly greater than the predation rate in early embryogenesis (ANOVA, Sidak's inequality, $P < 0.05$).

Egg production by *C. epialti* varied with temperature and crab embryogenesis (Fig. 2). The total production of egg strings was significantly lower at 4°C than at the other temperatures (ANOVA, Sidak's inequality, $P < 0.05$). There were no significant differences in total egg production at 10, 15, and 20°C temperature regimes. However, significant differences were found in the timing of egg production between these temperatures (Fig. 2). At 10°C, females produced egg strings while feeding on eggs in the limb bud stage of development (EDS III, Shields, 1987). At 10 and 15°C, egg production and the number of reproducing females increased with crab egg development. At

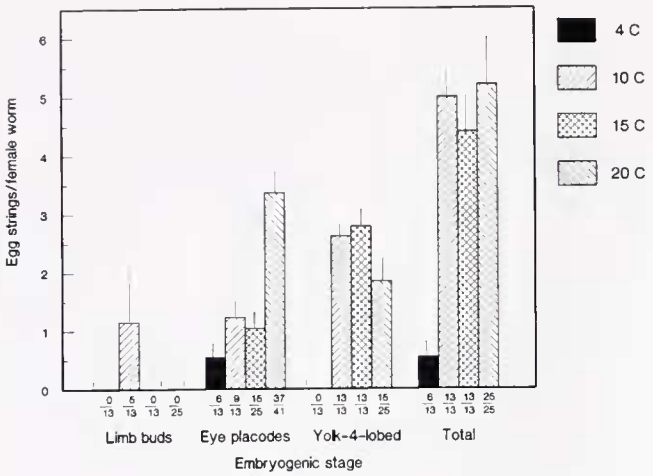

Figure 2. Egg production, measured as the number of egg strings produced per female *Carcinonemertes epialti*, in relation to temperature and crab embryogenesis. Numbers refer to egg-laying females and total number of females. Bars represent standard errors.

20°C, egg production and the number of reproducing females were highest in the eye placode stage of crab egg development (EDS IV, Shields, 1987), but these decreased significantly (ANOVA, Sidak's inequality, $P < 0.05$) in the four-lobed yolk stage (EDS VI, Shields, 1987).

Overall predation rates by the nemertean worms were not affected by the presence or absence of *L. callinectes*. However, changes in worm behavior were noted in those treatments containing the fungus. In general, when egg mortality was associated with the presence of the fungus, the worms no longer resided on the eggs or setae. Most often, the worms secreted new mucous sheaths directly onto the surface of the petri dish; yet some worms persisted on the infected eggs. Egg predation dropped only as a result of massive egg mortality caused by the fungus. *Carcinonemertes epialti* never ate eggs infected with *L. callinectes*, whereas eggs infested with bacteria were often preyed upon by the worm.

Lagenidium callinectes

Growth and attack rates of *L. callinectes* were affected by temperature and the different stages of egg development (Table I, Figs. 3, 4). At 4°C, no fungal growth was observed for any treatment during the experiment. At 10°C, *L. callinectes* had extremely low attack rates throughout the developmental period. The fungus infected significantly more eggs at 15°C than at 10°C, and infected significantly more at 20°C than at 15°C (ANOVA, Sidak's inequality, $P < 0.05$). Significantly fewer eggs were attacked during early embryogenesis than during middle and late embryogenesis at 15°C (Table I, Fig. 3: ANOVA, Sidak's inequality, $P < 0.05$).

The growth of *L. callinectes* was positively associated with initial zoospore density (Fig. 4). At low and moderate zoospore densities (15 and 150 zoospores/ml), the fungus attacked few eggs; subclinical infections were common (see below). At the high zoospore density (1500 zoospores/ml), fungal attack rates were significantly higher. These results must be interpreted with caution as the variances between treatments were not equal.

Temperature had a profound effect on the establishment and attack rates of the zoospores from the high initial density (Fig. 4). Significantly more dead eggs were found at 20°C than at 15°C in those replicates exposed to 1500 zoospores/ml ($T = 2.71$, $P < 0.02$).

Typically, *L. callinectes* grew either well or poorly in each replicate. The marked variation in the ability of the fungus to attack and kill eggs is shown by the large standard deviation and standard error in most treatments (Table I, Figs. 3, 4). Subclinical infections of *L. callinectes*, defined as infections not presenting fungal exit tubes and with few hyphae present, were observed in many replicates. These low level infections contributed to the high variation in attack rates. Subclinical infections killed few eggs during the ten-day trials. However, in longer trials (14+ days) these infections often became acute and produced higher death rates.

Lagenidium callinectes was never actually observed on any of the *Cancer anthonyi* collected in the Santa Barbara Channel. A *Lagenidium*-like fungus was observed in a few samples of crab eggs taken over the course of one year. The *Lagenidium*-like fungus had an overall prevalence of 2.6% (6/232 crab clutches). The fungus was found in three broods with eggs in early stages of development, two broods with eggs in mid or late stages of development, and one brood with eggs at eclosion. The fungus was not associated with high levels of crab egg mortality. Attempts to isolate this fungus failed.

No significant interactions were observed between the fungus and the bacteria. *Lagenidium callinectes* grew and attacked eggs at approximately the same rate regardless of the presence or absence of *Carcinonemertes epialti* or bacteria (Table I). However, *L. callinectes* often thrived in the presence of *C. epialti*. Fungal zoospores frequently attached to mucus produced by the worm and hyphae were occasionally noted on worm sheaths. Fungal growth was also noted frequently on crab eggs that had been damaged during the establishment of the experiment.

Bacteria

Bacteria were always present to some extent in the broods of *Cancer anthonyi*. Bacteria were never observed at high densities on the eggs of *C. anthonyi*. Egg mortality in the treatments containing bacteria and in control

Table I

Mean number of infected crab eggs (±SD) after ten days exposure to Lagenidium callinectes *at different temperatures*

Temperature	Treatment	Developmental stages of the eggs		
		Early	Middle	Late
10°C	F	2.6 ± 2.4 (5)	1.0 ± 1.7 (5)	0.0 (5)
	FW	0.8 ± 0.8 (9)	5.9 ± 3.7 (9)	0.4 ± 0.7 (9)
	FB	0.6 ± 0.9 (5)	1.6 ± 1.5 (5)	2.6 ± 3.2 (5)
15°C	F	3.7 ± 9.3 (20)	29.8 ± 35.9 (20)	32.6 ± 44.0 (20)
	FW	3.0 ± 2.8 (25)	12.0 ± 20.0 (29)	11.1 ± 16.3 (29)
	FB	6.0 ± 29.9 (5)	11.8 ± 15.7 (5)	30.2 ± 36.3 (5)
20°C	F	94.6 ± 32.8 (10)	79.2 ± 54.0 (9)	131.6 ± 75.2 (10)
	FW	125.9 ± 60.5 (9)	39.4 ± 48.6 (9)	129.5 ± 87.4 (9)
	FB	Not done	Not done	Not done

Treatments include presence of fungus alone (F); fungus and *Carcinonemertes epialti* (FW); and fungus and bacteria (FB). Numbers in parentheses represent the number of replicates per treatment. At 15°C, mortality in early stages of development was significantly less than mortality in later stages (ANOVA, Sidak's inequality, $P < 0.05$).

treatments was extremely low (Table II), and was not influenced by temperature.

Temperature and other sources of mortality

In some cases, egg mortality occurred in the absence of observed symbionts. In particular, temperature shock may have caused significant mortality (Table III, and see below). At 4°C, many eggs had swollen outer coats. By the end of the experiment, these eggs had died. Temperature shock may have also contributed to mortalities at 20°C. Asynchronous egg development was most apparent at 20°C (Table III) and to a lesser extent at 15°C. Asynchronous development was most notable in treatments containing eggs in late stages of development. Typically, some eggs developed normally, whereas other eggs developed at a much slower rate. Ovigerous crabs held at 15°C possessed few eggs in asynchronous development. Thus, experimental conditions may have contributed to the observed asynchronous development.

Temperature had a marked effect on embryogenesis of *Cancer anthonyi* (Table III; Fig. 5). Development proceeded more quickly at higher temperatures (15 and 20°C) than at lower temperatures. At 4°C, no visible egg development occurred. Interestingly, eggs held at 4°C for 10 days and then returned to 15°C renewed normal development. The duration of each developmental stage

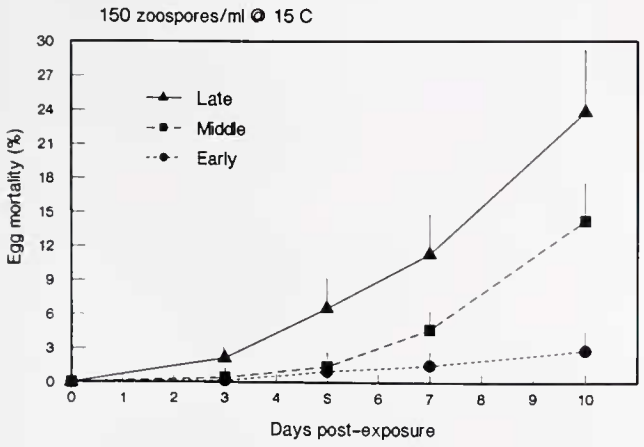

Figure 3. Growth of *Lagenidium callinectes* on different developmental stages of *Cancer anthonyi* eggs at 15°C. Growth was measured as a function of egg mortality. Twenty replicates per treatment. Bars represent standard errors.

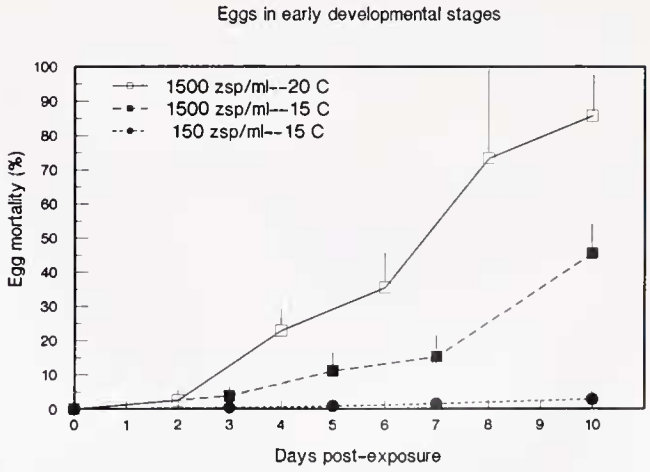

Figure 4. Growth of *Lagenidium callinectes* at different zoospore densities on the eggs of *Cancer anthonyi*. Twenty replicates per treatment, except for the 20°C treatment (5 replicates). Bars represent standard errors.

Table II

Mortality rates after ten days for bacterial treatments (B) and for the control treatments (C-untreated control, C_{aa}-bleach, antibiotics and agar control)

Temperature	Treatment	Developmental stages of the eggs		
		Early	Middle	Late
10°C	B	0.6 ± 0.7 (10)	Not done	0.5 ± 0.7 (10)
	C_{aa}	0.4 ± 0.5 (5)	0.0 (5)	0.0 (5)
	C	0.0 (5)	0.0 (5)	0.4 ± 0.5 (5)
15°C	B	0.6 ± 0.9 (5)	0.0 (5)	0.0 (5)
	C_{aa}	0.6 ± 1.0 (10)	0.7 ± 1.1 (10)	0.4 ± 1.0 (10)
	C	0.8 ± 0.6 (10)	0.1 ± 0.3 (10)	0.1 ± 0.3 (10)
20°C	B	0.6 ± 1.3 (5)	0.2 ± 0.4 (5)	0.0 (5)
	C_{aa}	0.5 ± 0.7 (5)	0.6 ± 0.8 (10)	0.1 ± 0.3 (10)
	C	0.4 ± 0.9 (5)	0.4 ± 0.8 (10)	0.5 ± 0.7 (10)

Numbers in parentheses represent replicates per treatment. There were no significant differences between treatments within temperatures.

became proportionally accelerated with increasing temperature (Fig. 5). At 20°C, considerable variation was noticed in the rate of development of eggs from different clutches. Accelerated development, wherein eggs developed at a much faster rate, was evident in some replicates at this temperature. It occurred primarily in eggs in late developmental stages, especially in eggs progressing from the eye placode stage to the yolk band stage (EDS IV–VI, Shields, 1987).

A protistan fungus, *Rhizophydium littoreum* (Chytridiomycetes), was isolated from field samples and some experimental treatments. Experimental exposures *in vitro* indicated that although the chytrid was associated with dead eggs, it was not a causal mortality factor (Shields, unpub. data).

Discussion

This study implicates *Carcinonemertes epialti* as the primary cause of egg mortality of *Cancer anthonyi*. In the presence and absence of other symbionts, these worms caused substantial egg mortality *in vitro*. The high prevalence (>97% of the ovigerous crabs were infested, Shields, 1987) and the relatively high predation rates of female worms at various temperatures suggest that these egg predators contribute most to egg mortality on individual crabs.

Outbreaks of nemertean egg predators on *Cancer magister* (Wickham, 1979a, b), *Hemigrapsus oregonensis* (Shields and Kuris, 1988), *Homarus americanus* (Aiken

Table III

Observations on the effects of temperature on egg mortality and abnormal or asynchronous development of Cancer anthonyi *embryos*

Temperature	Temperature related mortality			Asynchronous development		
	Early	Middle	Late	Early	Middle	Late
4°C	++	++	++	No development		
10°C	—	+	—	—	—	—
15°C	—	—	—	±	±	±
20°C	++	±	+	++	++	++

Key to mortality and development symbols: ++, present in large proportion of replicates, and to large degree in individual replicates; +, present in small proportion of replicates; ±, infrequently observed in replicates; —, not observed in replicates.

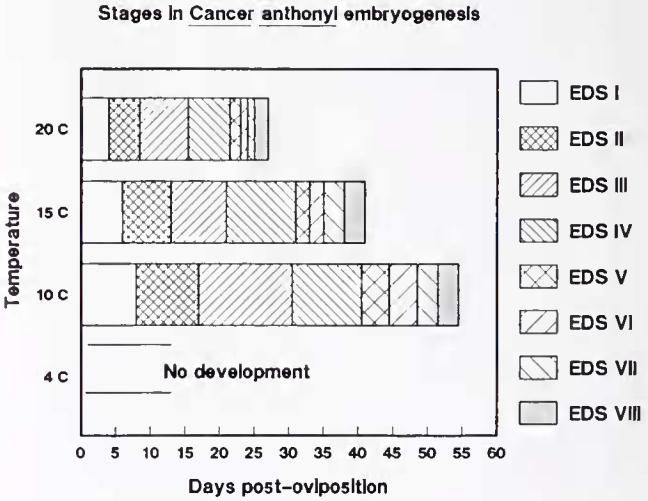

Figure 5. Development of *Cancer anthonyi* eggs as a function of temperature. EDS represents the developmental stage of the egg as per Shields (1987). EDS I represents eggs in one-cell stage through blastulation; EDS II, gastrulation through development of tagmata; EDS III, limb buds evident through buds well developed; EDS IV, development of eye placodes and eyes, faint heartbeat in live eggs; EDS V, completed eyes, yolk in 4 distinct lobes, strong heartbeat in live eggs; EDS VI, yolk reduced to a band or two lobes; EDS VII, yolk reduced to two small masses; EDS VIII, hatching. Data from composite results.

et al., 1985; Campbell and Brattey, 1985), and *Paralithodes camtschatica* (Wickham *et al.*, 1985, and in prep.) are associated with massive host egg mortality. The impact of these outbreaks on the reproduction of the individual host and host populations may be quite high (Wickham, 1979a, b; Wickham *et al.*, 1985; Kuris and Wickham, 1987; Shields and Kuris, 1988). While *Carcinonemertes epialti* has never been found at high, outbreak densities on *Cancer anthonyi*, it is clearly capable of causing the moderate levels of egg mortality observed on that host species.

Predation rates and aspects of reproduction of *Carcinonemertes* spp. have been examined (Wickham, 1979a, 1980; Roe, 1984; Kuris and Wickham, 1987). The feeding rates of mature male and female *C. epialti* at 15°C agreed with those observed by Roe (1984) at 12–14°C (males—1.3 eggs/day, females—2.4 eggs/day). No significant differences were reported between the predation rates of *C. epialti* and *C. errans* (Roe, 1984). Egg production—the total number of egg strings produced—averaged 3.1 egg strings/female for *C. errans* on *Cancer magister* (Wickham, 1980), 2.8 and 5.6 egg strings/female for *C. epialti* on *Hemigrapsus oregonensis* (Roe, 1979, 1984, respectively), and approximately 5.0 egg strings/female for *C. epialti* on *Cancer anthonyi* (this study). The predation rate of both *C. epialti* and *C. errans* increases with the onset of worm maturation and reproduction, and is closely timed with the development of the crab eggs (Roe, 1979; Wickham, 1979a, 1980; Shields 1987). This pattern was not affected by temperatures above 10°C. Worms ate and matured faster at higher temperatures, but total egg production was not significantly greater at elevated temperatures.

The timing of egg production by *Carcinonemertes epialti* was affected by temperature. The faster onset of reproduction in females at 10°C compared to that at higher temperatures may be explained by the timing of crab embryogenesis. Crab eggs developed faster at higher temperatures, hence worm reproduction occurred on eggs in later stages of development. Since the initiation of oviposition by *C. epialti* is closely tuned to crab embryogenesis (Roe, 1979; this study), and since crab embryogenesis may be greatly affected by temperature (Fig. 5), then the faster onset of reproduction and egg laying by *C. epialti* at 10°C may enable larval worms to hatch prior to and during host eclosion. Timing is important because female *Cancer anthonyi* strip the empty egg shells and associated debris (including worm egg strings) from the pleopods within 48 hours of crab egg eclosion (Shields and Kuris, in prep.).

In the present study, *Lagenidium callinectes* was not an appreciable cause of egg mortality except at the relatively high temperature of 20°C (ambient temp., 16°C). The fungus could cause massive mortalities (20–75% egg mortality) under certain conditions (high zoospore densities, high temperatures), but it was never observed on the eggs of *Cancer anthonyi* from nature. The *Lagenidium*-like fungus found on the crab eggs was not associated with egg mortality.

Lagenidium callinectes was originally described from the eggs of the blue crab, *Callinectes sapidus*, by Couch (1942). It has been reported from a variety of larval crustaceans (Sandoz *et al.*, 1944; Bland and Amerson, 1973; Lightner and Fontaine, 1973; Nilson *et al.*, 1975), including zoeae of *Cancer magister* (Armstrong *et al.*, 1976). The prevalence of *L. callinectes* on egg masses of *Callinectes sapidus* ranged from 0–87% of the ovigerous crabs (Rogers-Talbert, 1948). Typically, only the periphery of the egg mass was affected. Heavy infections were found in 25% of the ovigerous crabs (Rogers-Talbert, 1948). Infestation rates of *L. callinectes* were closely paralleled by those of *Carcinonemertes carcinophila* and egg mortality (usually less than 25%) fluctuated with these symbionts. Other studies have also found a relationship between the abundance of nemertean egg predators and the presence of fungal or bacterial symbionts (Wickham, 1979b; Miller and Fleming, 1983).

The physiology and nutrition of *Lagenidium callinectes* has been examined in detail (Bahnweg and Gotelli, 1980; Bahnweg and Bland, 1980). Several strains of *L. callinectes* have been isolated and their growth at different temperatures has been documented (Bahnweg and Bland, 1980). The L-1 strain of *L. callinectes* grew best at 17°C (Bahnweg and Bland, 1980), and, in the present study, it grew well at 20°C. Growth was delayed at 15°C (Rogers-Talbert, 1948). Subclinical infections were more common at lower temperatures and lower exposure levels. The 3–4 days required for the infection to spread to other eggs (Rogers-Talbert, 1948) was noted in the 20°C exposures but not at the lower temperatures. Thus, temperature had a marked effect on growth, development, and attack rates of *L. callinectes* on the eggs of *Cancer anthonyi*.

Temperature appeared to have little, if any, effect on bacterial growth and pathogenicity. Bacteria (*Leucothrix* sp. and others) contributed little to egg mortality in this study. Epibiotic bacteria are frequently found on the eggs of decapod crustaceans (Johnson *et al.*, 1971; Nilson *et al.*, 1975; Fisher and Wickham, 1976; Tharp and Bland, 1977). The role of bacteria as pathogens causing egg mortality is controversial. Bacteria have been implicated as sources of egg mortality in *Cancer magister* (Fisher, 1976; Fisher and Wickham, 1976) and *Homarus americanus* (Fisher *et al.*, 1978). Our study, controlling for the presence of other symbionts, demonstrates that bacteria are a negligible cause of egg mortality in *Cancer anthonyi*. A similar conclusion was reached for the eggs of *H.*

americanus (Harper and Talbot, 1984) and *Palaemon macrodactylus* (Fisher, 1983).

Few interactions were noted between symbionts. Nemerteans did not eat fungus-infected eggs, and they only occasionally resided on infected eggs. The worms ate eggs that were both coated and not coated with bacteria. *Lagendium callinectes* was occasionally found on the mucus, and mucous sheaths produced by *C. epialti,* and in this respect, the presence of nemerteans aided the establishment of the fungus.

Temperature plays an important role in the embryogenesis of many crustaceans (see Perkins, 1972; Wear, 1974; Amsler and George, 1984). In general, the development of *C. anthonyi* embryos was similar to that of other crustaceans. However, the embryos of *C. anthonyi* do not undergo diapause as do the embryos of *C. pagurus* (Wear, 1974). Wild (1983) found that the broods of *Cancer magister* hatched earlier at higher temperatures (17°C) than those at lower temperatures. He also showed that hatching success was inversely correlated with temperature. Curiously, hatching success was low at the ambient temperature (13°C). Mayer (1973) observed that *C. magister* embryos experienced massive mortalities at 15 and 20°C after only 3–6 days, whereas, few died at 5 and 10°C after over 11 days. *Cancer anthonyi* has a wider range of thermal tolerance because embryos survived for over 10 days at 4 and 20°C. The more southerly range of *C. anthonyi* compared to that of *C. magister* (Nations, 1975) may account for the successful development of *C. anthonyi* embryos at warmer water temperatures. Further, the lower thermal tolerance of *C. magister* embryos compared to that of *C. anthonyi* may explain why *C. anthonyi* is not abundant in northern California (Toole, 1985).

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